EVALUATION OF REPELLENT EFFECT OF EXTRACTS OF *Hyptis suaveolens* AND *Ocimum gratissimum* LEAF ON DIPTERA (CULICIDAE).

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ABSTRACT

The use of plant for mosquito repellents can serve as a reliable means to curb the problems of mosquito transmitted diseases and the resistance from synthetic repellents. The repellent activity of adult mosquito to extracts of n-hexane, acetone, ethanolic and 70 % methanol of *Hyptis suaveolens* and *Ocimum gratissimum* leaf were tested using World Health Organization (WHO) standard methods. Repellence was tested by exposing twenty five adult mosquitoes to extracts for 0.5 to 24 h. The extracts of the non polar solvents were more effective as repellents. Repellence by extracts of n-hexane *O. gratissimum H. suaveolens* leaf was highest over a period of 2 to 4 h than that of the extract of n-hexane *H. suaveolens* leaf. The standard repellent diethyl toluamide (DEET) gave 100 % repellent effect for a period of 4 h while 0 % repellent was recorded for the 24 h period. The *H. suaveolens* and *O. gratissimum* leaf therefore have the potential to be used in formulating mosquito repellents that are of lower cost and with lesser risk properties which will further benefit the local economy.

Keywords: mosquito, n-hexane, *Hyptis suaveolens*, *Ocimum gratissimum*, repellent

INTRODUCTION

Mosquitoes are insects of public health importance that constitute a great nuisance in addition to being vectors of dangerous and deadly disease pathogens (Baraack *et al*., 2018, CDC, 2019, WHO, 2017). Many of these mosquito borne diseases, especially malaria presents an obstacle to national development because of its high human and economic cost. Malaria imposes substantial costs on both individuals and governments. It results to low productivity at work; reduce physical growth and economic turnover (Fang, 2010, WHO, 2017). These havocs by mosquitoes are preventable through the control of mosquitoes will greatly spare man especially children and pregnant women the ordeal of morbidity and mortality. The most efficient mosquitoes disease prevention and control of mosquitoes population strategy is by the integrated vector management (IVM). These can be accomplished using combination of methods including habitat modification, pesticides (indoors and out door residual spraying), biological-control agents, trapping and genetically modified mosquitoes, (CDC, 2018; WHO, 2012) The control of mosquito rather than the treatment of the diseases caused by mosquito appears to be a quicker, better and cheaper control option. The commonly used chemical insecticides and repellents currently require a replacement or supplement (chemical or biological) due to the rapid resistant and toxic side effects (Oliver *et al*., 2010). DEET (N,N-Diethyl-meta-toluamide or diethyltoluamide) is the most efficient synthetic chemical substance used as mosquito repellents all over the world, however have controversy over it toxicity and resistance (Moore *et al*., 2007). The yellow fever mosquito has been reported to have developed a resistance to DEET and thereby not repelled by it. The synthetic pyrethroids (permethrin) also possess rapid insecticidal and repellent

action (Moore *et al.*, 2011). The capacity of mosquitoes to develop resistance has been shown to be hereditary (Stanczyk *et al*., 2010). Also their reported toxicity against the skin and nervous system can cause rashes, swelling, eye irritation and other serious problems to children. Hence it is strongly believed that bio-based natural mosquito repellents would be preferred over chemical mosquito repellents (Dhara *et al*, 2018).

The promising potential of plant derived compounds for insecticides and repellents formulations to eliminate the quick resistance development common to synthetic and non-biodegradable insecticides and repellents is worth exploring (Maia & Moore, 2011; Dhara *et al*, 2018). Botanical insecticides and repellents generally have specie and target specificity and are readily biodegradable, they also have lower bioaccumulation and low mammalian toxicity (Maia & Moore, 2010; Mi, 2018). Plant sourced repellents are good for the environment and also have added benefit of having good aroma. The role of natural mosquito repellent is to mask human scent due to the l-octen-3-ol, lactic acid and CO_2 which are present in the sweat and breath is responsible for attraction of mosquitoes towards human beings. The -chemoreceptors present in the antennae of mosquitoes perceive the smell of the sweat (Patel *et al*., 2012). Most essential volatile oils extracted from several plants have demonstrated topical repellents activity against mosquitoes (Nerio, *et al*., 2010). The components present in the oil responsible for the repellent characteristics provide only a short-lasting protection for less than three hours (Tawatsin *et al.*, 2001). The smell characteristic of the essential oil comes from the presence of plant components such as, citronellal, eugenol, geraniol, α-pinene and limonene (Dhara *et al*, 2018; Soleimani-Ahmadi *et al*., 2017).

Lamiaceae family of plants including *Hyptis* species (bushmint) and *Ocimum* species (basil) are found worldwide especially in the tropics and are locally used as repellent, fumigants, by rubbing on the skin, hanging on walls, burning of leaves to provide the only economically viable form of personal protection against mosquitoes in the local communities of developing countries including Nigeria (GBIF, 2016; Mann, 2012, Tropico, 2016).

This study aimed at testing different solvent leaf extracts of *Hyptis suaveolens* and *Occimum gratissimum* for mosquito repellency towards the prevention and control of mosquitoes transmitted diseases of public health significance and malaria in particular.

METHODOLOGY

Through ethnobotanical study *Hyptis suaveolens* and *Ocimum gratisimum* (the plants reported to be used as a mosquitocide and repellent) were collected from a location in Minna, Nigeria in the month of October. The plant botanical name was confirmed at the National Institute of Pharmaceutical Research Idu (NIPRID), FCT, Nigeria. Plant vouchers were deposited at the NIPRID herbarium with Voucher number NIPRID/H/6558 for *Hyptis suaveolens* and NPRID/H/6557 for *Ocimum gratisimum*.

The *Anopheles funestus* species of mosquitoes were employed for this study. The larvae that emerged from the eggs were maintained on a 3:1 ratio (w/w) of fish feed and yeast powder, while the adult were maintained on 10 % sucrose (WHO, 2012).

The leaf of *Hyptis suaveolens* and *Ocimum gratisimum* plants were washed under running tap water drained, sorted out and dried at room temperature for five days. It was powdered with electric blender and extracted by reflux using solvents of varied polarity (1:5 w/v) for 2 hours. Each of the resulting extracts was concentrated using a rotary evaporator at high pressure and reduced temperature (55 ^{o}C). The remaining solvent in the extract was allowed to evaporate at room temperature. A 10 % stock solution each of n-hexane, acetone, ethanol and 70 % methanol extracts of *Hyptis suaveolens* and *Ocimum gratisimum* leaf extracts were prepared by dissolving 1 g of crude extract in 9 ml acetone (Edeoga *et al*., 2006). From the stock 0.2, 0.4 and 0.8 % concentrations in olive oil were impregnated on filter papers that were able to fit on the WHO testing tube lid while mixture of solvent used for extraction and acetone was introduced on the control filter paper. Three testing tubes were set up and 25 adult mosquitoes were introduced into the middle tube and allowed to acclimatize for 30 minutes after which the lids separating the control and testing tubes were removed and the number of mosquito in each compartment was recorded after 0.5, 1, 2, 4 and 24 hours respectively. A combination of the two plant extracts for each solvent was also tested for repellency.

$$\% \text{ Repellence} = \frac{\text{Number of test adult mosquito repelled} - \text{Number of control mosquito repelled}}{} \times 100$$

RESULT

Table 1 displays the repellence activity (%) of leaf extract of *H. suaveolens* and *O. gratissimum* on adult *Anopheles funestus* mosquitoes for 4 hrs. The *H. suaveolens* plant, gave the highest repellence activity (%) with n-hexane leaf extract for 0.5 h while 70 % methanol leaf extract gave the lowest activity. The *O. gratissimum* n-hexane leaf gave the highest repellence for 4 hours while the 70 % methanol leaf

extract gave the lowest. The repellence activity of *O. gratissimum* plant was significantly higher than the repellence activity of *H. suaveolens* plant but not significantly different from the reference repellent (DEET). The repellent activities of the two plants were significantly different ($p<0.05$) compared to the control but not with the standard.

Table 2 presents the repellent activity (%) of the combined leaf extracts of *H. suaveolens* and *O. Gratisimum f*or each solvent. The highest repellence activity (100 %) was observed for the combined 1:1 ratio of the n-hexane leaf extract of *H. suaveolens* and *O. gratisimum*. The lowest repellence effect was obtained for the combined ratio 1:1 of 70 % methanolic leaf extract of *H. suaveolens* and *O. gratisimum*. The repellence activity of the combined n-hexane leaf extract was significantly higher ($p<0.05$) compared to the repellent activities of the other solvent leaf extracts and the control while it was not significantly different from the standard (DEET).

Figure 1 presents the repellence activity (%) of different solvent leaf extracts of *H. suaveolens* and O. *gratisimum* for adult mosquitoes after 24 h. The highest repellence activity (%) was observed for the n-hexane 1:1 combination ratio of *H. suaveolens* and O. *gratissimum* leaf extracts. The 70 % methanol leaf extract of *H. suaveolens, O. gratissimum* and DEET (standard repellent) gave 0 % repellence after 24 h. The repellence activity of the n-hexane, acetone and ethanolic leaf extract of *O. gratisimum* were not significantly different from each other but was significantly different for the methanol leaf extracts of both plants.

DISCUSSION

Repellence activity shown by the n-hexane, acetone and ethanol leaf extracts of *H. suaveolens* and *O. gratisimum* suggests that there are active principles responsible for repellence activities present in the plant extracts. This may be is accounted for by the reported confirmed presence of thymol, caryophyllenes, phytol and terpeneol in the plant extracts which have repellent activity (Adefolalu *et al.*, 2015). Comparing the repellence activity of O. *gratisimum* and *H. suaveolens* extracts, suggest O. *gratisimum* extracts have higher repellence substances than the *H. suaveolens* extract indicating that there may be a higher level of the different types of phytochemicals responsible for repellence activity in each plant are present.

The extracts of both plants maintained repellence activity for up to 24 h. it is important to note that the extracts from the present study appears to have better repellent compared to the commercial and other plant based repellents since it still maintained repellence after 24 h. The reason for this suggests that the phytochemical present in these extracts may be less volatile than those reported by other researchers. Also the plants have phytochemicals that are able block the odour sensing ability of the mosquito chemoreceptor despite the aromatic nature of the plants.

The standard repellent diethyl toluamide (DEET) gave 100 % repellent activity within 4 h but 0 % repellence after 24 h. The repellent time of the plant extracts in this study on the average peaked between the 0.5 to 4 h which is higher than that reported by previous findings. An average repellent protection time of essential oil is reported to be between five minutes and two hours (Fradin, 1998). Pushpanathan *et al*., (2008) reported 100 % protection against *Cx. quiquefasciatus* for 15, 30, 60 and 120 mins for the skin repellent essential oil from *Zingiber*

Officinalis. Repellence has been attributed to the presence of essential oils such as the eugenol, linalool, geraniol in the leaf of plant extracts. The short repellence duration may probably be as a result of the volatile nature of the chemical compounds responsible for the repellence. This was in agreement with the report of Tawatsin *et al.*, (2001), who stated that plant based repellents tends to be very volatile. Also they have shorter duration of action than synthetic compounds such as DEET (Maia & Moore, 2011). Plant based repellents and insecticides are also known to develop resistance less rapidly than the synthetic repellents and insecticides. The difference in repellence time may also be due to species, part of plant used, solvent of extraction, origin of the plant, photo sensitivity of plant compounds (Rawani *et al.*, 2009). The best lures or repellents may be cocktails of multiple compounds (Patel, 2016).

The import of this finding is that these extracts can further be improved and developed into natural based repellent products which could become more acceptable, affordable and used for personal protection against mosquito. The use of fixative agents such as vanillin, liquid paraffin, and salicylic acid can increase the repellent efficiency of substances with repellent potentials (Songkro, 2012; Mi, 2018). These plant repellents also have higher possibility to be used and applied directly on the skin unlike permethrin which is recommended to be used as an insect repellent on clothing only and not directly on the skin. Permethrin-treated clothes can be effective against insects for 2 weeks to 6 months even after 5–20 detergent washings (Diaz, 2016). The established usefulness of the *Hyptis and Occimum* plants species commonly used as food suggest it possible safety (Edeoga *et al.*, 2006), compared to DEET, PMD (p-menthane-

3,8-diol from the leaves of lemon *eucalyptus*), citronella, clove oil, permethrin (from *Chrysanthemum cinerariifolium*) and other plant with reported eyes, nose, skin irritation, toxicity and carcinogenicity concerns (Diaz, 2016; Yoon 2015).

CONCLUSION

The n-hexane and acetone leaf extracts of *H. suaveolens* and *O. gratissimum* had active ingredient with repellence activity over a period of 2 to 24 h comparable to the reference repellent (DEET). *O. gratissimum* had higher percentage repellence than *Hyptis suaveolens* leaf extracts. The plant extracts can formulated with stabilisers and synergists for improved repellency, long-lasting protection, and enhanced safety. Further research could be carried out to isolate and determine the mode of action of some of the phytoactive compounds that are present in the plants.

Table 1: Repellence activity (%) of leaf extracts of *Hyptis suaveolens and Ocimum gratissimum* on adult *Anopheles funestus* mosquitoes

Time (h)	n-Hexane	Acetone	Ethanol	70 % Methanol	DEET
H.suaveolens					

0.0	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
0.5	67.85±1.50[d]	47.20±2.80[c]	63.30±1.50[d]	60.15±0.30[c]	100.00±0.00[b]
1.0	28.55.0±2.00[c]	57.50±0.55[d]	53.30±2.30[c]	63.30±1.30[d]	100.00±0.00[b]
2.0	25.00±1.20[c]	32.40±3.90[b]	55.70±1.55[c]	59.55±0.09[c]	100.00±0.00[b]
4.0	18.75±6.25[b]	52.25±2.15[d]	28.55±8.75[b]	16.65±1.50[b]	100.00±0.00[b]
O.gratissimum					
0.0	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
0.5	94.40±5.60[b]	100.00±0.00[d]	93.50±0.20[d]	54.60±2.60[c]	100.00±0.00[b]

1.0	100.0±0.00^{c}	100.00±0.00^{d}	84.70±2.35^{bc}	65.25±5.25^{d}	100.00±0.00^{b}
2.0	100.0±0.00^{c}	93.20±0.50^{c}	79.20±6.50^{b}	45.85±1.50^{b}	100.00±0.00^{b}
4.0	100.00±0.00^{c}	81.80±1.20^{b}	97.05±0.50^{d}	46.35±0.25^{b}	100.00±0.00^{b}

Values are mean of three determinations and ± standard error of mean. Values in the same column with different superscript are significantly different ($p<0.05$).

Table 2: Repellence activity (%) of combined leaf extracts of *Hyptis suaveolens* and *Ocimum gratissimum* on adult *Anopheles funestus* mosquito

Time (h)	n-Hexane *H.suaveolens/ O.gratissimum*	Acetone *H.suaveolens/ O.gratissimum*	Ethanol *H.suaveolens/ O.gratissimum*	70% Methanol *H.suaveolens/ O.gratissimum*	DEET
0.0	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
0.5	95.80±2.20[c]	82.85±2.50[d]	66.65±3.30[d]	57.15±0.30[de]	100.00±0.00[b]
1.0	100.0±0.00[d]	76.90±7.70[dc]	70.00±2.00[d]	54.50±1.18[d]	100.00±0.00[b]
2.0	100.0±0.00[d]	86.20±0.50[c]	64.55±2.50[c]	53.00±0.09[d]	100.00±0.00[b]
4.0	96.15±2.60[c]	67.85±1.85[c]	93.75±1.25[e]	41.60±1.81[c]	100.00±0.00[b]

Values are mean of three determinations and ± standard error of mean. Values in the same column with different superscript are significantly different ($P<0.05$).

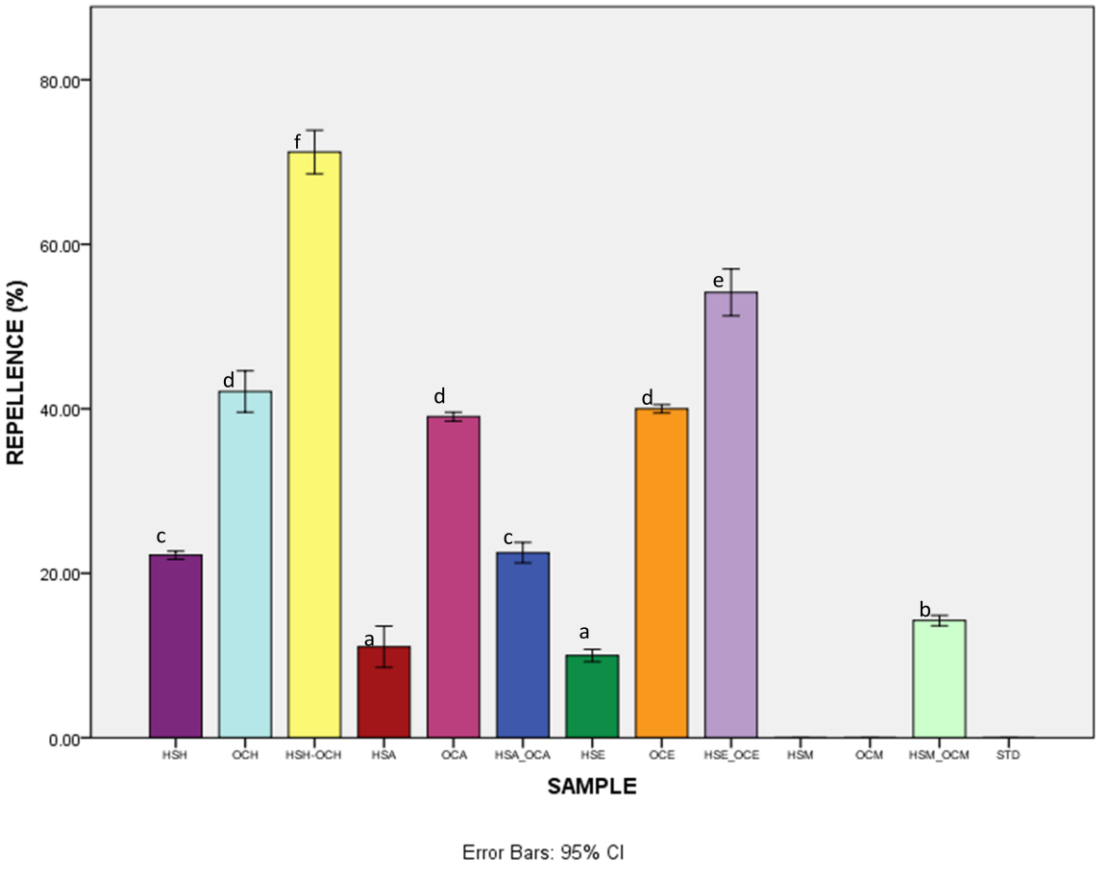

Figure 1: Repellence activity (%) of different leaf extracts of *Hyptis suaveolens* and *Ocimum gratissimum* on Adult *Anopheles funestus* mosquito at 24 h.

Key:

HSH –*Hyptis suaveolens* n-hexane leaf extract

OCH- *Ocimum gratissimum* n-hexane leaf extract

HSA- *Hyptis suaveolens* Acetone leaf extract

OCA- *Ocimum gratissimum* Acetone leaf extract

HSE- *Hyptis suaveolens* Ethanolic leaf extract

OCE- *Ocimum gratissimum* Ethanolic leaf extract

HSM- *Hyptis suaveolens* Methanolic leaf extract

OCM- *Ocimum gratissimum* Methanolic leaf extract

STD- Standard

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